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Diploschistes tianshanensis sp. nov., a corticolous species from Northwestern China

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Abstract—*Diploschistes tianshanensis* is described as a new species, found on rotten wood with mosses and other lichens (*Cladonia* sp.) in an arid region of Northwestern China. The new fungus, which is diagnosed by urceolate ascomata and large ascospores, resembles *D. gypsaceus* but is readily distinguished by its epruinose thallus surface. ITS rDNA sequence analyses support the taxon as a distinct species. The description of *D. tianshanensis* is accompanied by notes on its chemistry, distribution and ecology, and comparison with related species.

Key words—Central Asia, biodiversity, *Graphidaceae*, *Ostropales*, taxonomy

Introduction

Diploschistes Norman includes crustose lichens with a blackish pseudoparenchymatous proper exciple, lateral paraphyses, and a trebouxioid photobiont (Lumbsch 1989, Lumbsch & Mangold 2007). The genus is widely distributed in arid and semiarid regions worldwide and comprises 35–44 species (Pérez-Vargas et al. 2012, Fernández-Brime et al. 2013, Abbas et al. 2014). Most *Diploschistes* species occur on rocks, some on soil, and a few species are rarely found on wood or bark (Lumbsch & Mangold 2007). After Lumbsch (1989) monographed Holarctic species, several papers on *Diploschistes* were published covering cladistic analysis and molecular phylogeny (Lumbsch & Tehler 1998, Martín et al. 2003, Fernández-Brime et al. 2013), ecology and distribution

(Pant & Upreti 1993, Guderley & Lumbsch 1996, Lumbsch & Elix 2003, Mangold et al. 2009), and taxonomy including new species and combinations (Lumbsch & Elix 1985, 1989; Lumbsch & Mayrhofer 1990; Lumbsch & Aptroot 1993; Lumbsch & Mangold 2007; Pérez-Vargas et al. 2012; Abbas et al. 2014). The genus exhibits a remarkably variable morphology of the ascomata, which vary from perithecioid to urceolate and lecanoroid (Lumbsch 1989, Lumbsch & Mangold 2007). Despite this variation, *Diploschistes* is currently regarded as monophyletic and accommodated within the *Graphidaceae* (*Ostropales*) based on molecular studies (Frisch et al. 2006, Martín et al. 2003, Fernández-Brime et al. 2013).

The lichen biota of Northwestern China is rich, with more than 670 species (in 127 genera) previously reported (Abbas & Wu 1998, Guo 2005). Nevertheless, lichen diversity is still incompletely known in the region and new species can be expected to continue to be described. Of the nine *Diploschistes* species recorded from China, four were described from the Northwestern region (Wei 1991, Guo 2005, Abbas et al. 2014).

An additional *Diploschistes* species recently collected in Xinjiang, Northwestern China, is described here as *D. tianshanensis*, based on morphological and chemical characters. The ITS region of nrDNA of the type specimen has also been sequenced and compared with GenBank sequences to assess the phylogenetic affinities of the new species.

Materials & methods

The specimens studied were collected from Mountain Tianshan, Xinjiang, China, and deposited in the Herbarium Mycologicum Academiae Sinicae-Lichenes, Beijing, China (HMAS-L) and the Lichen Section of Botanical Herbarium, Xinjiang University, Urumqi, China (XJU). Their morphology was examined using a Zeiss Stemi SV 11 stereomicroscope. Sections for anatomical examination were cut by hand using a razor blade, mounted in water, studied with a Zeiss Axioskop 2 plus light microscope, and photographed using a Nikon Digital Camera D50. Chemical constituents were identified by thin-layer chromatography using solvent systems A, B and C (Orange et al. 2010).

DNA extraction, amplification, and sequencing. DNA was extracted from thallus fragments with ascomata of the type specimen using the DNAsecure Plant DNA Kit (Tiangen, China) following the manufacturer's protocol. The ITS region was amplified according to Martín et al. (2003) and Abbas et al. (2014). The entire ITS region (ITS1+5.8S+ITS2) of the nrDNA repeat tandem was targeted for the Polymerase Chain Reaction amplification using the primers ITS1 and ITS4 (White et al. 1990) and performed in a 25μL volume containing 0.75 units of TransStart Taq Polymerase (Tiangen, China), 2.5 μL of the buffer, 0.5 μL of a 5 μM solution of the primers, 2 μL of 2.5 mM for each dNTP solution, and 1 μL of genomic DNA. Thermocycling protocols

were 95°C for 3 min linked to 35 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min, with a final extension of 72°C for 10 min. The PCR products were screened on 1% agarose gels stained with ethidium bromide and sequenced by Genewiz Inc. (Beijing, China).

Newly obtained sequences were submitted to GenBank. The sequences were annotated according to Abbas et al. (2014). We excluded the 3′ end of the 18S (SSU) gene, and the 5′ end of the 28S (LSU) gene from the analyses. Sequences for the new species were complemented with similar *Diploschistes* taxa for which ITS sequences were available in GenBank (Table 1). Representative taxa were selected based on morphological characters, the results of Blast searches of ITS sequence, and the literature (Martín et al. 2003, Fernández-Brime et al. 2013, Abbas et al. 2014).

Table 1. GenBank accession numbers and voucher information for *Diploschistes tianshanensis* and 11 *Diploschistes* sequences downloaded from GenBank

Species	Voucher	GenBank
D. tianshanensis 1	China, Abbas 20s1	KC959951
D. tianshanensis 2	China, Abbas 20s2	KC959952
D. cinereocaesius	AFTOL-ID 328	HQ650715
D. diacapsis 1	Spain, BCC-Lich 13392	AF228317
D. diacapsis 2	Spain, BCC-Lich 13393	AF228318
D. gypsaceus	Switzerland, ESS-9047	AJ458284
D. gyrophoricus	Spain, BCC-Lich 11883	AJ458285
D. muscorum 1	Spain, BCC-Lich 13390	AF228319
D. muscorum 2	Spain, BCC-Lich 13391	AF229193
D. rampoddensis	Papua New Guinea, hb. Lumbsch	AJ458286
D. scruposus	Germany, ESS-21508	AJ458287
D. thunbergianus 1	Australia, hb. Lumbsch	AJ458289
D. thunbergianus 2	Australia, hb. Lumbsch	AJ458290

Phylogenetic analysis and sequence comparisons. The ITS sequences of the two examined samples and 11 selected representatives were aligned both by ClustalW and Muscle implemented in MEGA 5 (Tamura et al. 2011). The alignment matrix was realigned by StatAlign for reliable measurement of the accuracy of the results (Novák et al. 2008). The final matrix (submitted to TreeBase with accession number S14250) can be obtained from the corresponding authors.

The evolutionary history was inferred by using the Maximum Likelihood method (ML) based on the Tamura 3-parameter model in MEGA5 and Bayesian inference (PP) based on GTR model with rates = Invgamma. The analyses involved 13 nucleotide sequences. Absolute distances were also calculated in MEGA5, using the number of base differences between sequence pairs and with all gaps removed from each sequence pair.

Results & discussion

Sequence and phylogenetic analysis

The ITS1+5.8S+ITS2 region was successfully sequenced for the two Xinjiang samples, with the lengths varying by only one base pair (487–488 bp) for the entire region. The partial sequence containing SSU 3′ end and LSU 5′ end is included in the data submitted to GenBank.

A few positions in our sample sequences were difficult to align with the reference sequences and were excluded from the matrix. There were a total of 540 positions in the final dataset, all of which were used in the phylogenetic analyses.

The ITS sequences indicate that *D. tianshanensis* probably belongs to the *D. scruposus*-group (sensu Martín et al. 2003) with close affinities to the sub-cosmopolitan species, *D. diacapsis* (97% identity; 1% gap) and cosmopolitan species *D. gypsaceus* (96% identity; 1% gap) and *D. scruposus* (96% identity; 1% gap). The evolutionary history was inferred as the tree with the highest log likelihood (–1479.4326). The phylogenetic analyses strongly supported the monophyly of *D. tianshanensis* (ML = 100%; PP = 1) and *D. diacapsis* (ML = 97%; PP = 1) but only weakly supported (ML = 57%) the relationship

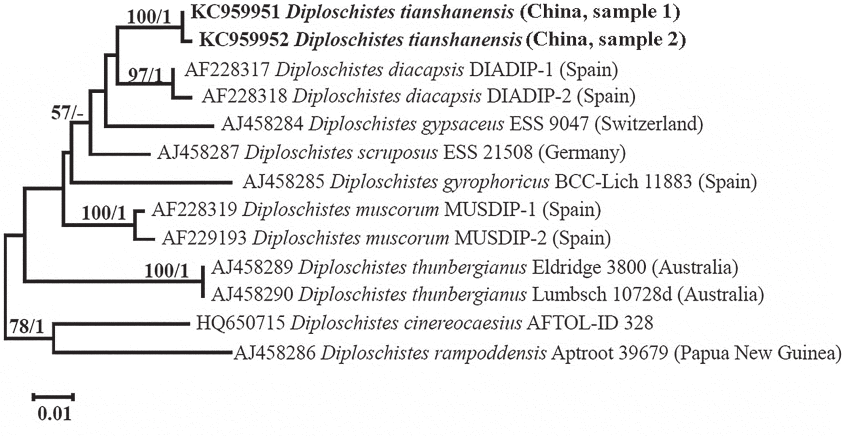

Figure 1. Phylogenetic relationships inferred from ITS sequences of *Diploschistes tianshanensis* and some closely related species in the *D. scruposus* group (with *D. cinereocaesius* and *D. rampoddensis* as outgroup). Support is indicated for branches with Maximum Likelihood bootstrap frequencies >50%, and Bayesian Inference posterior probabilities >0.95. The evolutionary tree was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model. The tree with the highest log likelihood (–1479.4326) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

between *D. tianshanensis* and its morphologically closely related species including *D. diacapsis*, *D. gypsaceus*, and *D. scruposus* (Fig. 1).

Absolute distances for the aligned sequences of the ITS region also support the separation of a new species. In our sequence matrix, distances between infraspecific samples are ≤3 while distances between species are ≥13 (Table 2).

Table 2. Absolute distances between ITS sequences from *Diploschistes tianshanensis* and closely related species (gaps ignored in pairwise comparisons).
Infraspecific distances are indicated with **bold font**.

	1	2	3	4	5	6	7	8	9	10
1. *D. tianshanensis* 1										
2. *D. tianshanensis* 2	**1**									
3. *D. diacapsis* 1	13	14								
4. *D. diacapsis* 2	15	16	**2**							
5. *D. gypsaceus*	17	18	17	19						
6. *D. scruposus*	16	17	14	14	15					
7. *D. gyrophoricus*	25	26	24	25	26	24				
8. *D. muscorum* 1	19	20	20	20	22	14	24			
9. *D. muscorum* 2	20	21	21	20	22	14	25	**3**		
10. *D. thunbergianus* 1	31	32	29	29	35	28	35	26	27	
11. *D. thunbergianus* 2	31	32	29	29	35	28	35	26	27	**0**

Taxonomy

Diploschistes tianshanensis A. Abbas, S.Y. Guo & Ababaikeli, **sp. nov.** Figure 2

MycoBank MB 804328

Differs from *Diploschistes muscorum* by its grayish-green thallus without pruina and its larger ascospores.

Type: China. Xinjiang: Urumqi Co., Tianshan Mountain, Bayi forest farm, 43°21′N 86°49′E, alt. 1760 m, on rotten wood of *Picea schrenkiana* Fisch. & C.A. Mey., 27 Aug. 2011, A. Abbas 110821 (**Holotype,** HMAS-L; **isotype,** XJU; GenBank KC959951, KC959952).

Etymology: The specific epithet *tianshanensis* refers to the region in which the type specimen was collected.

Thallus corticolous, crustose, continuous, whitish-grey to grayish-green, moderately thin, ≤0.7 mm thick. Upper surface dull, without pruina. Medulla white, amyloid (I+ blue). Photobiont trebouxioid with cells ≤16 μm diam. Prothallus not visible. Vegetative propagules absent. Ascomata apothecia, immersed. Disc urceolate, without pruina, orbicular, 0.5–1.0 mm diam. Proper exciple dark brown, 60–100 μm thick. Hymenium hyaline, 120–180 μm high, not inspersed. Hypothecium yellowish brown,

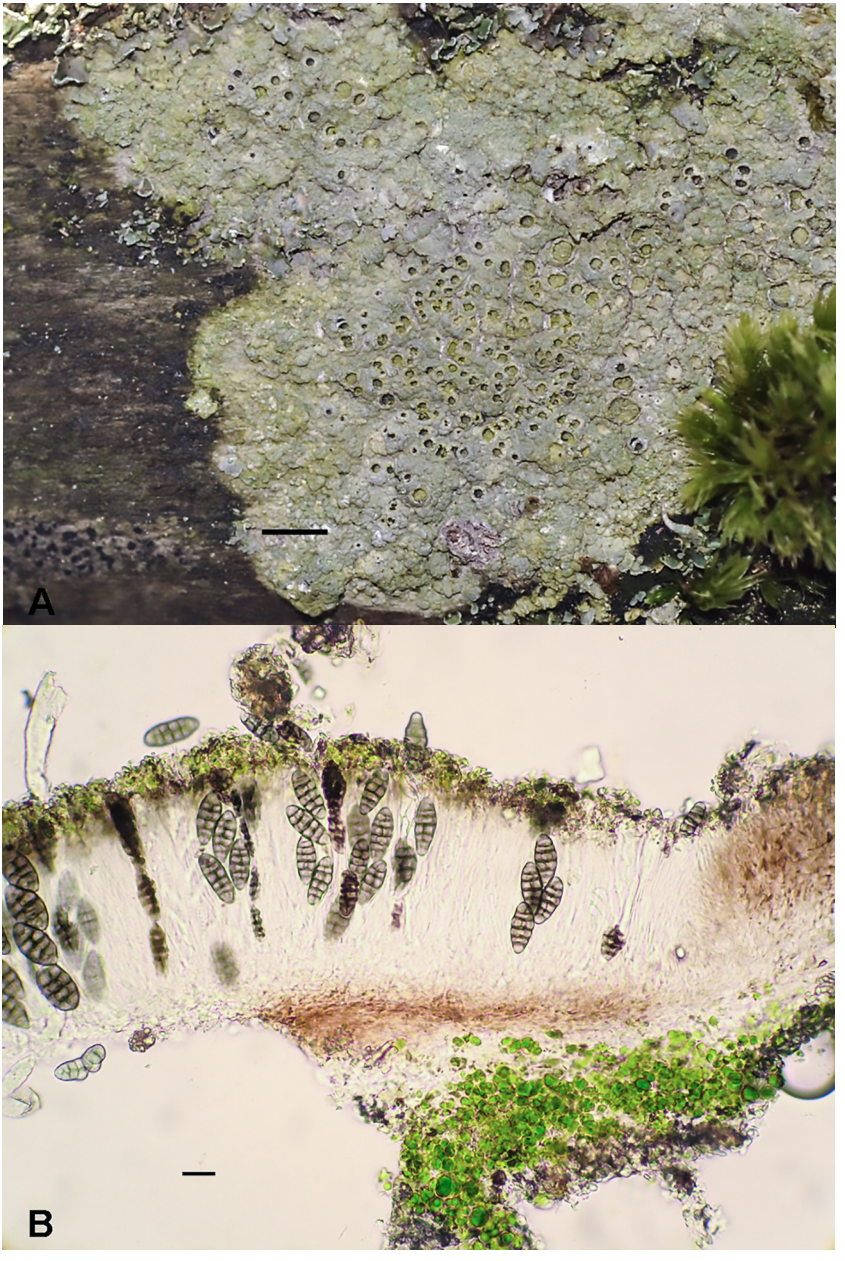

Figure 2. *Diploschistes tianshanensis* (holotype, HMAS-L Abbas 110821). A. habit; B. asci and mature ascospores. Scales: A = 2 mm; B = 20 μm.

15–30 µm thick. Paraphyses 1–2 µm thick, simple, apices not thickened. Asci cylindrical, 90–130 × 20–30 µm, 4-spored. Ascospores ellipsoid, brown, muriform, with 4–6 transverse and 1–2 longitudinal septa, 20–36(–42) × 12–18 µm. Pycnidia unknown.

Spot tests—K+ yellow, C+ and KC+ red, PD–.

Secondary metabolites—Diploschistesic and lecanoric acids detected (TLC).

Ecology—At present *D. tianshanensis* is known only from the type locality in Northwestern China. It grows on rotten wood of *Picea schrenkiana*, together with *Cladonia* sp. and mosses.

Additional specimen examined — **China. Xinjiang**: Urumqi, Tianshan Mountain, Bayi forest farm, 43°21′N 86°49′E, alt. 1800 m, 15 May 2010, A. Abbas 201000515 (XJU).

Comments —*Diploschistes tianshanensis* is characterized by its corticolous habit, grayish green appearance, absence of pruina, urceolate apothecia, large ascospores, and the presence of diploschistesic and lecanoric acids. To the best of our present knowledge, other *Diploschistes* species having a corticolous habitat include only *D. muscorum* (Scop.) R. Sant., which can be distinguished by its combination of 4-spored asci, juvenile parasitism, and the presence of grayish or whitish pruina. The new species resembles morphologically the cosmopolitan *D. gypsaceus* (Ach.) Zahlbr., which differs by its usual occurrence on calcareous substrata in shaded, damp habitats (Lumbsch 1988, Fletcher & Hawksworth 2009). *Diploschistes gypsaceus* has not yet been reported from China (Wei 1991, Guo 2005, Abbas et al. 2014).

Similar species also include *D. diacapsis* (Ach.) Lumbsch and *D. scruposus* (Schreb.) Norman. *Diploschistes diacapsis* is a terricolous species that is readily identified by its whitish pruinose thallus, (4–)8-spored asci, and the ascospore size (20–40 × 14–20 µm). *Diploschistes scruposus,* which is saxicolous but not corticolous, also has an epruinose thallus, (4–)8-spored asci, and may or may not contain diploschistesic acid. A comparison of the diagnostic characters of *D. tianshanensis* and morphologically similar species is provided in Table 3.

Previous studies that have used DNA sequence data for species recognition in lichens required both monophyly in single-locus ITS phylogenies and diagnosable morphological differences (e.g. Han et al. 2013, Abbas et al. 2014). Our morphological and molecular data for our new species meet the requirement for accurate species delimitation and support recognition of *Diploschistes tianshanensis* as a species new to science.

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Table 3. Diagnostic comparisons of *Diploschistes tianshanensis* and closely related species

Species	Pruina	Ascomata	Asci	Spores (µm)	Habitat	Acids	Distribution
D. diacapsis	+ (whitish)	Urceolate–lecanoroid	4–8-spored	20–40 × 14–20	Terricolous/calciferous	Lecanoric, diploschistesic, orsellinic	Sub-cosmopolitan
D. gypsaceus	+	Urceolate	4-spored	30–34 × 15–18	Calciferous	Lecanoric, orsellinic	Cosmopolitan
D. muscorum	+ (grayish/whitish)	Urceolate	4-spored	18–32 × 6–15	Corticolous/terricolous	Lecanoric, diploschistesic	Cosmopolitan
D. scruposus	–	Urceolate	4–8-spored	25–40 × 10–20	Saxicolous	Lecanoric, diploschistesic	Cosmopolitan
D. tianshanensis	–	Urceolate	4-spored	20–40 × 12–18	Corticolous	Lecanoric, diploschistesic	Central Asia

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